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Grain number and grain filling of two-row malting barley in response to variation in post-anthesis radiation: Analysis by grain position on the ear and its implications for yield improvement and quality.

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Abstract

Grain weight is reported to be a relatively well conserved characteristic across spring barley (*Hordeum vulgare* L.) crops that vary in grain number m^{-2}. Understanding the mechanisms that promote stability in grain weight is important to ensure that efforts to increase grain number beyond current high levels successfully increase yield without compromising grain quality. The aims of this study were to establish 1) whether post-anthesis grain abortion contributes to the stability of grain weight by helping match grain numbers to post-anthesis assimilate supply and 2) whether variations in post-anthesis assimilation per unit grain number affect the heterogeneity of grain weight. Field experiments were conducted in a high-yield potential environment for spring barley in 2011 and 2012. Crops were either shaded post anthesis (a 59% reduction in radiation incident on the crop) to reduce net carbon assimilation or grown unshaded. Grain growth was measured at different spikelet locations on the ear and on different shoots (main shoot and tillers) of the same plant. Shading crops from 14 days after anthesis until the harvest maturity reduced yield by 19-20%, mean grain weight (MGW) by 12-16% and harvest index by 5-6%, but did not significantly affect grain number in either year. The magnitudes of these effects were considerably lower than the reduction in radiation imposed by shading suggesting some compensatory adjustment in radiation use efficiency or dry matter partitioning to grain after shading. The rate of grain filling was higher for grains in central spikelets than grains at distal or basal locations on the ear. Shading reduced the rate of grain filling to a similar extent (23-27%) at most locations evaluated on the ear, but had no effect on the duration of grain filling. In spite of the comparable effects of shading on grain growth across different spikelet positions and hierarchy of shoots, crops harvested after shading tended to have a more variable individual grain weight (larger interquartile range and coefficient of variation) than crops that were unshaded. The results show that post-anthesis grain abortion does not contribute to the

stability of MGW in spring barley. Moreover, low levels of post-anthesis radiation in crops of large grain number m^{-2} (sink capacity) can increase heterogeneity of grain weight, which may have negative consequences for grain quality.

Keywords: barley, grain number, grain weight**,** heterogeneity, radiation, spikelet location

1. Introduction

Understanding the relationships between grain number formation, grain development and grain filling is fundamental to our efforts to increase cereal yields through plant breeding and improved crop management. Grain yield of barley, as with other cereals, is the product of two components, the number of grains produced per unit ground area and the mean grain weight (MGW; Gallagher et al., 1975). While grain number in barley varies widely with location and season and typically accounts for the majority of the variation in yield across environments (Gallagher et al., 1975; Baethgen et al., 1995; Abeledo et al., 2003; del Moral et al., 2003; Bingham et al., 2007; Peltonen-Sainio et al., 2007; Serrago et al., 2013; Kennedy et al., 2017), grain weight tends to be less variable and is poorly correlated with yield (Gallagher et al. 1975; Bulman et al. 1993; Baethgen et al., 1995; Abeledo et al., 2003; Sadras and Slafer, 2012). The smaller variability in MGW may be a consequence of evolutionary and/or breeding selection for increased grain size, as larger seed with larger embryos and storage reserves have a greater chance of producing seedlings that establish successfully, are able to compete with neighbouring plants and tolerate damage from herbivores (Sadras, 2007). At present, the physiological mechanisms that underlie this apparent conservation of MGW are not fully understood, especially in the context of the large variations in grain number. A relatively stable MGW implies that the number of grains set is in some way matched to the potential of the crop to supply assimilates for grain filling (i.e. the sink capacity is set lower

than the source capacity or that the sink and source capacities are maintained in relatively close balance) and that only in circumstances where post-anthesis assimilation is reduced significantly, for example by drought or disease, will grains fail to fill adequately.

There are several possible mechanisms through which this might be achieved. Firstly, grain numbers and grain storage capacity (potential grain size) may be determined concomitantly prior to fertilization according to some common measure of overall assimilate availability (Sinclair and Jamieson, 2006; Sadras and Denison, 2009). In this way an upper limit may be set on grain size and the numbers of grains adjusted in concert. There is ample evidence to suggest that the number of tillers and florets that survive to produce ears and grains respectively is regulated by assimilate availability or organ and crop growth rate during late stem extension (Gallagher et al., 1976; Hay and Kirby, 1991; Prystupa et al., 2004; Slafer et al., 2009; Sadras and Slafer, 2012). Similarly, potential grain size has been correlated with carpel size at anthesis which, in turn, is sensitive to treatments that vary carbon assimilation and crop growth prior to ear emergence (Calderini et al., 2001, 2006).

Secondly, the same outcome (a relatively conserved MGW) might be achieved if there was some mechanism for reducing the number of grains after anthesis if conditions during grain development and filling restrict the assimilation capacity. Adjustments in the grain number of barley and other small grain crops have been observed in studies evaluating post-anthesis treatments such as shading, temperature modification, and drought (Habgood and Uddin, 1983; Nicolas et al., 1985; Grashoff and d'Antuono, 1997; Zinselmeier et al., 1999; Boyer and Westgate, 2004; Boyer and McLaughlin, 2007; Estrada-Campuzano et al., 2008; Sanchez-Bragado et al., 2016). In many cases the mechanisms responsible for these losses have not been elucidated, although grain abortion has been reported for several species (Zinselmeier et al., 1999; Estrada-Campuzano et al., 2008). In maize, the abortion of grains induced by crop water stress can be prevented by the exogenous supply of sugars, suggesting

that a limited carbohydrate supply may be responsible for the abortion (Zinselmeier et al., 1999). Not only are grain numbers sensitive to reductions in assimilation, but the growth of surviving grains can also be affected differentially depending on their location on the ear. In wheat grain growth was reduced by post-anthesis shading to a smaller extent in florets located closest to the base of the rachilla compared to those further away, thereby increasing the variation in individual grain weight (Bremner and Rawson, 1978). Similarly grains in florets closest to the rachilla were least sensitive to increases in assimilate availability induced by partial degraining (Xie et al., 2015). Comparable data on the response of grains at different positions on barley ears are lacking.

The mechanisms by which grain numbers are matched to the potential supply of assimilate during grain filling and the way in which assimilate is allocated between grains, both within and between ears, has implications for how plant breeding might increase yield without compromising grain quality. Currently yield of barley is generally considered to be sink limited (Bingham et al. 2007a; Kennedy et al., 2017). A route to increase the yield of sink limited crops is, therefore, to increase grain numbers (Pedro et al., 2012; Reynolds et al., 2012; Miralles et al., 2000; Gonzales et al., 2003). However, if the sink capacity is expanded so that source and sink are brought into closer balance at the start of grain filling, the crop may be at greater risk of source limitation should environmental conditions subsequently deteriorate. Significant grain abortion in the face of increased source limitation could help maintain grain quality, but restrict yield improvement. Alternatively, if post-anthesis grain abortion is not an important mechanism in barley, the result of increased source limitation might be a reduction in grain quality associated with lower mean grain weight and possibly greater heterogeneity of individual grain weight. In spring barley heterogeneity of grain weight is undesirable for maltsters, because variable grain are more difficult to process (Passarella et al., 2003).

Questions about the regulation of grain numbers in response to post-anthesis assimilation and its potential consequences for yield and quality are best answered thorough a detailed analysis of grain formation and growth at specific spikelet positions on the ear as this provides a greater resolution than standard yield component analysis. The aim of the research reported here was to investigate the effects of varying post-anthesis assimilation, through shading, on grain growth of spring barley at discrete positions on ears of main shoots and primary tillers. The specific objectives were to 1) establish whether there is any evidence of grain abortion in response to a reduction in post-anthesis incident radiation and hence assimilation and 2) determine the effects of variations in radiation per unit grain number on heterogeneity of grain weight.

2. Materials and Methods

2.1 Site characteristics and experimental design

Field experiments were conducted on spring barley (*Hordeum vulgare* L., cv Quench) at Teagasc, Oak Park, Carlow, Ireland in 2011 and 2012. Quench is a two-row malting variety selected because of its popularity amongst growers at the time of the study. Its yield and grain quality characteristics were representative of other recommended varieties. In each year the fields were sheltered, relatively flat and located 52^{o} 51' N, 6^{o} 54' W at an altitude of 57 m. The top soil texture (determined by hand texture analysis) was loam (USDA, Rowell, 1994) with a moderate moisture holding capacity. The site was characterised by continuous arable production and the experiments occupied a position in the rotation that is standard practice for commercial spring barley production in the region. In 2011 the previous crop was winter barley and in 2012 it was winter wheat.

Crops were sown on 10th March 2011 and 14th March 2012 at a seed rate of 330 viable seeds m^{-2}. Shading and unshaded control treatments were allocated at random to plots to give a randomised block design with six replicates in 2011 and four replicates in 2012. Plot size in 2011 was 6 m^2 (2 m wide x 3 m long) with 2 m wide discard plots between shaded and control plots to avoid overshadowing. Shading treatments were applied to entire plots in 2011. Plot size in 2012 was 96 m^2 (4 m wide x 24 m long) and shades were erected over sub-plots of 2 x 3 m; here the shades were located alongside discard areas *within* plots to avoid overshadowing. Shaded and unshaded plots were further sub-divided into two sampling areas; one for destructive sampling of ears for grain growth assessment during the treatment period and one for final grain number, biomass and yield determination at harvest. These are referred to as the frequent and final sampling areas respectively.

2.2 General husbandry and imposition of shading

Crops were managed for high yield potential with the aim of keeping the crop well supplied with mineral nutrients and free of pests and disease. Nitrogen applications of 132 (2011) and 154 kg N ha^{-1} (2012) were split (50:50) between early post-emergence when tramlines became visible and during tillering. Maintenance applications of P and K were made after sowing based on soil chemical analysis. Fungicides were applied shortly before stem extension and at ear emergence. Applications of aphicide and herbicide were as required.

Shading treatments were imposed 14 days after Zadoks growth stage (GS) 55 (50% ear emergence; Tottman and Broad, 1987). As anthesis in spring barley tends to occur before the ear is fully emerged this timing also corresponded to approximately 14 days after anthesis (GS 61). The timing of shading was selected to avoid potential interference with the fertilization of grains, particularly on later developing tiller ears. Shades were left in place until after grain physiological maturity (GS 87). The shading material used was an open

weave black polystyrene shade-netting (Tildenet Ltd., Bristol, UK) erected on a frame of fencing posts and rope at a height of 1.1 m above ground level. Simultaneous measurements of photosynthetically active radiation (PAR) above the crop canopy under the shades and in adjacent unshaded areas were made using a Sunscan Canopy Analysis System (Delta T Devices, Cambridge, UK). Shading reduced incident PAR by 59% averaged across replicate plots.

2.2 Microclimate and non-destructive measurements of crop growth

A pyranometer (SPLite2, Kipp and Zonen B. V., Delft, Netherlands) and a relative humidity/temperature probe (MP100A, Rotronic Instruments (UK) Ltd., Crawley, UK) connected to a data logger (CR1000, Campbell Scientific Ltd., Loughborough, UK) were installed in shaded and unshaded treatment areas in 2011 to monitor environments hourly for solar radiation, relative humidity and temperature. In both 2011 and 2012, soil was sampled to 30 cm using a Dutch style auger in shaded and unshaded plots at the end of the shading period to determine the gravimetric soil moisture content of the upper profile (Rowell, 1994). Crop height was measured throughout the shading period in the undisturbed final sampling areas of treated and untreated plots or sub plots by measuring the height of five randomly selected shoots from ground level to the uppermost leaf ligule or ear collar (if present). The percentage green area of whole treatment areas was estimated by visual assessment at approximately weekly intervals during the latter stages of canopy senescence in shaded and unshaded treatments. On each occasion a single plot score was estimated based on the combined area of leaf laminae, stems and ears, including awns. Plots were inspected for leaning and lodging at each visit to the site and its occurrence recorded if observed. The severity was assessed just prior to harvest by estimating the % area affected in each of the following five categories: shoots upright; shoots leaning slightly (0-5° from the vertical); shoots leaning (5°-45° from

the vertical); shoots lodged (45°-90° from the vertical); brackled (stem failure a 1/4 or more up its length).

2.3 Destructive sampling and measurements

Grain weight was assessed at individual grain locations, or zones, on ears of control and shaded plants at the beginning of the treatment period and again at harvest in both seasons. Additional weekly assessments were carried out during the treatment period in 2011. These detailed grain weight assessments were carried out on the main stem ear (MS) only in 2011, but also on two subsequent tiller ears (T1 and T2) in 2012. In 2011, ten main stem ears per plot were sampled at random from the designated frequent sampling area on each sampling occasion (main stems had been tagged with a small wire ring prior to the onset of tillering so that they could be identified from the primary tillers). In 2012, ten plants were sampled from the designated sampling area and the MS, T1 and T2 were identified based on their growing position at the plant base. Decreasing stem diameter, height and ear length were also used as indicators of tiller order if growing position was not clear. There was at least 0.5 m distance between adjacent sample areas and sampling was avoided within 0.5 m from the ends and edges of plots/treatments to avoid edge effects. Tram lines and drill overlaps were also avoided with the aim of selecting sample areas that were representative of the plot. After sampling ears were stored in sealed plastic bags at 4-6^{o}C for a maximum of 24h prior to sampling of individual grains.

The central spikelet on each ear was identified by counting the number of spikelets (fertile and infertile) upwards from the ear collar (alternating from one side of the ear to the other), halving the total number, and then rounding up to the next whole number. Grains were then sampled individually by their location relative to the central spikelet, with grains at the central spikelet designated as occupying zone 0, consecutive grain locations above (distal to

the central spikelet) designated +1, +2 etc. and grains below (basal to the central spikelet) designated -1, -2 etc. Cultivar Quench is a two-row barley variety where only the median spikelets at a given node on the rachis are fertile (Kirby and Appleyard, 1984). A spikelet was defined as possessing a 'grain' once it had swollen to twice the width of the two lateral infertile spikelets or if it had developed an awn and was not sampled unless it satisfied these criteria. The grains were sampled by removing bulk florets (including lemma, palea and awn) from each zone location and combining grains from a given zone from all 10 ears. The number of grains per zone was also counted so data would provide an accurate estimation of grain number ear^{-1}. Material was then dried at 70°C for 48h (or to a constant mass) before the dry weight was recorded to 0.1 mg.

At harvest ripeness, a quadrat (0.72 m^2) of above ground crop material was sampled at random from the previously designated final sampling areas and was air-dried prior to processing. A 40% sub-sample (by shoot number) was obtained for above ground dry matter determination and a further 20% was separated into ears and straw. Ears were counted and tissue fractions dried at 70°C for 48h for dry weight determination. Ears were then hand-threshed between two pieces of foam board and sieved over a mechanically operated 1.0 mm slotted sieve (Glasbläserei, Institute for Fermentation and Biotechnology, Berlin, Germany) to separate into chaff and grain portions. Material was re-dried before the dry weight of each portion was recorded. MGW was calculated for each plot using an automated grain counter (Pfeuffer GmbH, Kitzingen, Germany) by counting the number of grains in an approximate 25 g grain sample. After counting, grain weight was determined to the nearest 0.1 mg. Hand threshed grain yield (t ha^{-1}) was then expressed at 100% dry matter, grain number m^{-2} calculated as yield divided by MGW (expressed at 100% dry matter), and grain number per ear calculated as grain number m^{-2} divided by ear number m^{-2}.

Distribution of individual grain weight was measured for shaded and unshaded treatments in 2011 from the hand threshed grain samples at final harvest. Samples were poured onto a tray, mixed well (samples were not shaken prior to pouring to avoid stratification of grain size) and spread across the tray. Working from one end of the tray to the other one hundred grains per replicate were then removed and weighed individually to 0.1 mg.

To test whether hand threshed grain samples contained some non-viable grain, imbibed caryopses of defined size class were stained with 2,3,5 triphenyltetrazolium chloride (TTC) (Sigma-Aldrich Co. UK). Samples were taken at harvest ripeness from an accompanying field experiment conducted on cv Quench at a neighbouring site in 2011 in which plots were sown at seed rates ranging from 40 to 1280 seeds m^{-2}. This provided grain samples with a wide range of MGW. Other than the contrasting seed rates, the plots were grown under the same husbandry regime as that described above. Air dried ears from a sample of 6 x 1 m row lengths were counted and subsampled (20%) before hand-threshing and sieving over a 1.0 mm slotted sieve as described above to separate chaff from grain. Grains were then sieved over a 1.75 mm slotted sieve to separate suspected non-viable grains from the grain sample. All suspect non-viable grains (i.e. those not meeting the criteria used to define a grain when sampling by location on the ear; above) passed through the 1.75 mm sieve. The resultant 1.0-1.75 mm size class consisted of approximately 30-60 suspected non-grains and grains. These were imbibed in distilled water for 18h at 20^{o}C before removing from the water and cutting longitudinally to expose each half of the caryopsis. Tissue was then stained with 1.0% v/v TTC for 3h at 30^{o}C in the dark. Viable embryo tissue was stained red and was distinct from the non-stained endosperm. Grains (with viable embryo) and non-viable grains were separated, counted and dried at 70^{o}C for 48h and weighed. The % of non-viable grains in the sample, per unit ground area and per ear were calculated.

2.4 Statistical analysis

Harvest yield, yield component and biomass data obtained from the harvest ripe quadrat samples were analysed statistically for effects of shading using one way anova in GenStat (14th Edition, VSN International Ltd., Hemel Hempstead, UK). Grain number per ear data from the detailed grain weight assessments were similarly analysed. In 2012 an additional early shading treatment was included in the experimental design. All treatments were included in the statistical analysis to increase its power, but only the treatments of relevance to this paper (i.e. shading from GS55/61 +14 days and controls) are presented here. Results from the early shading treatment will be included in a following publication. Following ANOVA, relevant means for treatments of interest were compared using the standard error of the difference (SED) between means, on the residual degrees of freedom (df) from the ANOVA, thus invoking the least significant difference (LSD) at the P=0.05 level of significance. Estimations of grain heterogeneity at harvest were conducted by box-plot analysis of individual grain weight for each treatment in 2011 after first pooling data from each replicate plot and removing those in size classes <5.0 mg. Mean grain weights and coefficients of variation were then calculated for each replicate plot and differences between shading treatments tested by one-way analysis of variance.

Repeated measures ANOVA was carried out on data from detailed grain weight assessments at harvest in each year. Grain location within an ear was used as the repeated measure to account for possible correlations between grain weight at different spikelet positions. Main stem data for 2011 were first analysed to determine shading, zone (grain location), and shading x zone interaction effects on grain weight at harvest. Due to missing values at ear extremities (MS ears varied in their number of spikelets and grains per ear) data were restricted to zones +14 to -11. Split-line regression analysis (Genstat 14th Edition, VSN International Ltd., Hemel Hempstead, UK) was utilised to evaluate the relationship between

grain weight and time after GS55/61 and to estimate the rate and duration of grain filling for grains at selected locations on the ear. A bilinear model with a plateau was fitted following Miralles et al., (1996) as:

$$Y = a + bx, \text{ if } x<c; \qquad Y = a + bc, \text{ if } x \geq c;$$

Where Y is grain dry weight; a is the Y-intercept; b is the slope (the rate of grain filling); x is the time after GS55/61 (taken to be time after anthesis) and c is the effective duration of grain filling.

Repeated measures ANOVA was used to analyse effects of shading in 2012 on individual grain weight at harvest by position on the ear and by tiller hierarchy. All data were checked for normal distribution of residuals and equality of variance to ensure it conformed to the assumptions of parametric analysis.

3. Results

3.1 Climatic conditions and crop development

Plots established well in each year; the percentage plant establishment from the seed rate of 330 seeds m^{-2} was 89 % and 96 % in 2011 and 2012, respectively. The 2011 season was characterised by having a warmer and drier than average spring (March and April), but a cooler and drier August, whilst solar radiation was close to the long term average for the whole growing season (Fig. 1). By contrast, spring was cooler in 2012, and the summer considerably wetter. The levels of solar radiation in 2012 were lower than the long term average for April, June and July.

The mini-meteorological stations installed in shaded and unshaded plots in 2011 showed that the temperature just above the canopy was on average 0.4°C cooler in the shaded plots than the unshaded plots and the relative humidity was on average 0.3 % higher (hourly data

averaged across the whole shading treatment period). Gravimetric soil moisture content in the 0–30 cm profile at the end of the shading period in 2011 was 21% for the shading treatment and was significantly greater than the unshaded control value of 15 % ($P< 0.001$); there was no difference in 2012. Shading had no significant effect on crop height in either season (data not shown). The reduction in PAR incident at the top of the canopy due to shading was consistent on each measurement occasion, with average reductions of 59% in 2011 and 2012.

Shading tended to delay canopy senescence with the % green area declining to <10% around four or five days later than controls in both 2011 and 2012 (Fig. 2). In 2011 shading increased the % of stems that brackled from 2% to 12% ($P=0.002$) and those that leaned slightly from 1% to 4% ($P=0.038$) (data not shown). Although leaning (5-45^{o} from the vertical) and brackling occurred in 2012 (7% and 6% respectively on average across treatments) there was no significant difference between shaded and control plots ($P>0.05$). Leaning and brackling occurred after grain maturity and thus will have had negligible effect on yield.

3.2 Yield and yield components

Shading reduced yield by 20% in 2011 and 19% in 2012 ($P<0.05$; Table 1). In each case this was associated mostly with a reduction in MGW (16 and 12% for 2011 and 2012 respectively; $P<0.05$). By comparison the effects on grain number m^{-2} were small (5-8%) and not statistically significant ($P>0.05$). Neither of the two components of grain number (ear number m^{-2} and grain number ear^{-1}) were significantly affected by shading although there was a reduction in ear number m^{-2}, of comparable size in each year (5-11%), that accounted for the lower grain numbers m^{-2} (Table 1). Total above ground biomass and harvest index were also reduced ($P<0.05$) by shading in each year. Again the magnitude of the effect was comparable in 2011 and 2012.

Detailed grain weight assessments made at harvest on MS ears from 2011 and MS plus T1 and T2 ears in 2012 (Table 2) also showed that shading had no effect on grain number ear^{-1} in either season. There was a significant effect of shoot hierarchy on grain number ear^{-1} ($P<0.001$) where tillers had fewer grains per ear than the MS, but this was not accompanied by a shading x shoot interaction effect, implying that grain number of shoots of contrasting hierarchy did not differ in sensitivity to shading.

3.3 Grain growth at different locations on the ear.

There were more grains formed above the central spikelet of main stem ears than below it, as a greater number of the basal spikelets immediately above the collar were infertile. At harvest ripeness, grains in central spikelet locations (between +6 and -6) were heavier than those in more distal and basal positions in both the unshaded control and shaded plots in each year (Fig. 3). The decline in grain weight beyond these positions was steeper towards the base of the ear than it was towards the apex, with variability in grain weight at a given location greater at the distal and basal positions than the central positions. In 2011 there was an overall effect of shading ($P<0.001$) and grain location ($P<0.001$) on final MS grain weight, but no shading x grain location interaction ($P = 0.23$).

When a repeated measures analysis was conducted for 2012 data, including shoot hierarchy as a factor, shading was found to reduce grain weight at harvest ($P<0.001$) compared to unshaded controls, (32.7 mg vs 37.9 mg), but there were no significant interactions between shading and shoot hierarchy or grain position ($P>0.05$). This indicates that shading had the same effect on grain weight regardless of the type of shoot the ear was on or the position of the grain on the ear. There was a significant shoot hierarchy effect on grain weight ($P<0.001$) where the main stem (MS) MGW of 38.9 mg (averaged across shading treatments and grain position) was greater that of tiller 1 (T1) and tiller 2 (T2)

MGW's of 34.0 and 33.0 mg, respectively. There was also a significant shoot hierarchy x grain position interaction ($P<0.001$; Fig. 4). Thus grain weights in central locations on the ear were comparable in MS, T1 and T2 ears, but differences were observed in distal and, to a greater extent, the basal positions. At these locations, grains on the MS were heavier than those on T1 and T2 ears (Fig. 4).

The weight of grains at all locations on the MS ear increased steadily throughout the grain filling period under shaded and unshaded conditions in 2011, until reaching a plateau at the end of grain filling (representative grain positions shown in Fig. 5). A split-line regression fitted the data well with an R^2 >0.82 at all grain positions (with the exception of the most basal location analysed) with and without shading (Table S1). At spikelet location -11 the R^2 was lower (0.46-0.49; Table S1). The effects of shading on final grain weight were associated with a 23-27% reduction in the rate of grain filling at all positions except the most basal where the reduction was smaller (10%). By contrast the effects of shading on the duration of grain filling were generally small and not statistically significant (comparison of 95% CI with the difference between means). The duration of grain filling was around 43-46 days regardless of shading and over a wide range of grain positions. The smaller final weight of grains located at the extremities of the ear was associated with a decline in the rate of grain growth away from the central region.

3.4 Effects of shading on heterogeneity of grain weight

The frequency distributions of individual grain weight in quadrat samples at harvest in 2011 revealed a tail of very light material in the weight classes 0-2.5 and 2.5-5.0 mg (data not shown). Shading did not affect ($P>0.90$) the proportion of harvested grains below 5.0 or 2.5 mg in 2011 (Fig. 6). Vital staining using TTC showed that non-viable grains in hand threshed samples had an average weight of 2.9 mg; grains heavier than this were viable. Values for the

weight class <5.0 mg were, therefore, removed from the data set as non-viable grains and the variability in weight of remaining (viable) grains analysed. Grain harvested from shaded crops in 2011 had a lower median (36.8 mg) and a wider variation of weights (50% of grains within a 13.1 mg range from 29.8-42.9 mg) than grain from unshaded crops (median weight 43.6 mg; 50% of grains within a 10.3 mg range from 38.2-48.5 mg; Fig. 7). In these samples with the light weight fraction removed, mean grain weight was 16% lower ($P<0.001$) with shading than in unshaded controls and the mean coefficient of variation was increased from 20.5% to 27.5% ($P<0.001$).

4. Discussion

There was no evidence from the analysis of yield components or detailed grain growth within ears of a significant reduction in grain number ear^{-1}, ear number m^{-2} or grain number m^{-2}, in response to shading, suggesting that following fertilisation and early development, grains are unlikely to abort even if subject to large reductions in assimilate availability. Also, the absence of a significant shading x shoot interaction effect on grain number ear^{-1} in 2012 indicates that a grain abortion mechanism was not more likely to occur on later formed tillers. These conclusions are supported by the observation that the number of grains in the light weight fractions (<5mg), which included non-viable and empty grains, were not increased by shading. The grain numbers achieved for the unshaded treatments in 2011 and 2012 of 22,347 and 20,335 grains m^{-2}, respectively, reflect crops with a very large sink capacity, even compared to average high-yielding crops from similar environments (Kennedy et al., 2017). As such, assimilate availability per grain in the absence of shading was already likely to be at the lower limit of what is normally experienced in the field, yet despite a 59% reduction in photosynthetically active radiation for an extensive post-anthesis period there was still no significant reduction in grain number per ear compared to unshaded controls.

These findings are in contrast to results of some of the previous studies in barley and other crops where significant reductions in grain number were observed following a decrease in post-anthesis assimilation capacity (Habgood and Uddin, 1983; Nicolas et al., 1985; Westgate and Boyer, 1986; Grashoff and d'Antuono, 1997; Zinselmeier et al., 1999; Boyer and Westgate, 2004; Boyer and McLaughlin, 2007; Estrada-Campuzano et al., 2008). Post-fertilisation abortion of ovaries in maize following water deficits has been shown to be triggered by the depletion of ovary sugar pools (Zinselmeier et al., 1999; Boyer and Westgate 2004; Boyer and McLaughlin, 2007). Furthermore, shading of bread wheat crops post-anthesis resulted in significant reductions in grain number for crops in Mexico (Sanchez-Bragado et al., 2016). However these water deficit and shading treatments were applied closer to pollination than the shading treatment in this study. Florets are particularly sensitive to environmental stress during meiosis (nuclear and cell division in preparation for anthesis; Kirby and Appleyard, 1984) and thus environmental stress during early reproduction can result in floret sterility or decreased grain set in addition to post-fertilization grain abortion (Nicolas et al., 1985; Saini and Westgate, 2000; Fabian et al., 2011). Given that anthesis does not occur simultaneously across all plants, ears, and spikelets of field grown barley it was reasoned that commencing the shading treatment 14 days after anthesis (when the date of anthesis is assessed as an average across all shoots) would allow all potential grains to be fertilised prior to shading. In such a scenario any down regulation of grain number m^{-2} in response to post-anthesis shading could be attributed to a post-anthesis abortion rather than non-fertilisation. When water deficits were applied to maize for a period similar to the shading period here, grain weight was reduced, but there was little effect on grain number (McPherson and Boyer, 1977; Jurgens et al., 1978). This is in line with the present study and suggests that grain abortion in response to a restricted assimilate availability is more likely to occur closer to anthesis. However, it must be noted that shading from as late as 8 days after

anthesis reduced grain number ear^{-1} in wheat (Sanchez-Bragado et al., 2016). These results suggest that the sensitivity of grain number to variation in early post-anthesis PAR may differ between species.

Post-anthesis shading was reported to increase heterogeneity of grain weight in barley and reduce grain malting quality (Grashoff and d'Antuono, 1997), but the cause of the greater heterogeneity was not investigated. Our results show that shading reduced grain weight at all grain locations on the ear. With the exception of one basal spikelet location examined where the variability in grain weight over time was high, the lower grain weight with shading was the result of a slower rate and not a shorter duration of grain growth. The rate of grain filling was reduced by shading to a comparable extent at all spikelet positions and there was no evidence of an early arrest of grain growth at any particular location. These results and the lack of a significant interaction between grain location and shading on final grain weight indicates that when supplies were limited, assimilates were not partitioned preferentially to the central grains at the expense of the more distal and basal grains in either in main shoot ears or tillers. This would be expected to limit any increase in heterogeneity of grain weight when conditions restrict post-anthesis assimilate availability. It is clear, however, that these effects are not absolute as there was an increase in the coefficient of variation for grain weight of bulk samples after shading. In wheat, growth of grains was also reduced by shading more or less equally in different spikelet positions, but growth of florets at different positions within spikelets was altered unequally (Bremner and Rawson, 1978). The results were interpreted in terms of variation in the vascular connections between spikelets and between florets within spikelets and its consequences for the resistance of phloem translocation pathways. Increased heterogeneity of grain size and weight is undesirable in spring barley, because it is associated with variable protein concentrations and inconsistent germination in the malting process (Yin et al., 2002; Passarella et al., 2003).

It is important to recognize that, in addition to reducing incident radiation, shading can alter other aspects of the microclimate around crops. However, differences in temperature and relative humidity between shaded and unshaded environments were small and, therefore, the effects of shading on grain filling and yield were unlikely to be a consequence of changes in meteorological conditions other than incident radiation (Fisher, 1985). Although the cooler temperature under the shading might be expected to extend the duration of grain filling (longer calendar period for the same thermal time) the difference was only 11.9^{o}Cd (assuming a base temperature of 0^{o}C) over the period from the start of shading to the end of grain filling. This represents less than a day in the current study and is within the margin of error associated with determination of the grain fill duration (Fig. 5, Table S1). Shading did not reduce the top soil moisture content relative to unshaded controls in either season indicating that shading structures did not obstruct rainfall to the crop.

Proposals for increasing yield by increasing the number of grains formed rest on the premise that 1) yield of spring barley is sink-limited and that additional assimilate can be made available to meet the demand of a larger grain number and 2) grain numbers can be increased without reducing their potential storage capacity (potential grain weight). The magnitude of the source-sink imbalance in typical production environments will dictate the extent to which grain numbers can be increased before effects on grain filling occur. Indeed, there is evidence that in some production environments barley yield may be source rather than sink-limited (Alvarez Prado et al., 2013). As our results indicate that grain numbers are not reduced in response to reductions in post-anthesis radiation, crops whose source and sink capacities are in relatively close balance at anthesis could be at greater risk of poor grain filling and a greater heterogeneity of grain weight in those environments where restrictions to post-anthesis assimilation are commonplace (e.g. in response to drought or dull wet weather). There is evidence, however, that photosynthesis and RUE during grain filling of wheat can be

upregulated in response to an increase in sink demand (Reynolds et al., 2005). In the present study reducing radiation interception per unit grain number through shading also appeared to result in some compensatory adjustment in assimilate supply to the grain. The reduction in radiation interception (59%) greatly exceeded the reduction in yield (19-20%) and MGW (12-16%) and similar observations have been made by other authors from shading experiments (Willey and Holliday, 1971; Grashoff and d'Antuono, 1997; Arisnabarreta and Miralles, 2008a; Serrago et al., 2013). The delayed onset or slower initial rate of canopy senescence in shaded plots may have contributed to this, although the duration of grain filling was not increased appreciably and rapid grain filling ceased before complete canopy senescence. There may have also been increases in RUE and utilization of storage reserves for grain filling under shading, but these were not measured in the current study. It remains to be seen whether similar adjustments are also possible in barley if radiation interception per grain is reduced through an increase in grain number rather than decreased incident radiation.

5. Conclusions

Our results show that large scale reductions in post-anthesis incident radiation did not promote grain abortion in spring barley. Thus post-anthesis grain abortion is not an important mechanism for promoting stability of grain weight in barley when assimilate availability is restricted. Mean grain weight was reduced and the heterogeneity of grain weight increased. Final grain weight was reduced to a similar extent at the majority of spikelet locations on the ear through effects on the rate rather than duration of grain filling. This response limits, but does not prevent, an increase in heterogeneity of grain weight in barley when incident radiation is reduced. Further, the reduction in grain weight was considerably less than the reduction in incident radiation, reflecting possible compensatory adjustments in RUE and assimilate partitioning to the grain. Efforts to increase yield through increases in grain

numbers could have negative effects on grain size and malting quality depending on the extent of the source-sink imbalance of the crop and its capacity for increasing the supply of assimilates for grain filling when the sink capacity is raised.

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Table 1. Mean values of yield, yield components and other harvest variables for crops of spring barley treated with post-anthesis shading or unshaded in 2011 and 2012.

	2011				2012			
	Unshaded	**Shaded**	**SEM**	**P-value**	**Unshaded**	**Shaded**	**SEM**	**P-value**
Yield (t ha^{-1}; 100% DM)	9.35^{a}	7.48^{b}	0.41	0.025	6.8^{a}	5.5^{b}	0.25	0.026
Grain number m^{-2}	22347	21266	1192.6	0.550	20335	18685	802.5	0.253
MGW (mg; 100% DM)	41.9^{a}	35.4^{b}	0.71	0.001	33.3^{a}	29.3^{b}	1.02	0.033
Ear number m^{-2}	1208	1156	55.3	0.531	997	892	34.0	0.168
Grain number ear^{-1}	18.4	18.4	0.29	0.947	20.4	21.0	0.93	0.623
Harvest Index %	59.9^{a}	56.1^{b}	0.55	0.005	50.9^{a}	48.4^{b}	0.60	0.004
Ear number plant^{-1}	4.1	3.9	0.27	0.531	3.1	2.8	0.11	0.168
Total biomass (t ha^{-1} DM)	15.7^{a}	13.7^{b}	0.50	0.039	13.4^{a}	11.2^{b}	0.48	0.049

Means with different letter superscripts are significantly different ($P<0.05$); for 2012 based on Fishers least significant difference test. DM = dry matter; MGW = mean grain weight.

Table 2. Effects of post anthesis shading on spring barley grain number per ear at harvest for main shoots (MS) in 2011 and main shoots, tiller 1 (T1) and tiller (2) in 2012. Within a column means followed by a different letter are significantly different at ($P<0.05$) based on Fisher's LSD

	Grain number ear^{-1}	
	2011	**2012**
Post-anthesis shading		
Unshaded	24.4	22.0
Shaded	24.2	21.7
Shoot hierarchy		
MS	-	24.3^{a}
T1	-	21.4^{b}
T2	-	19.9^{c}
S.E.M.	0.38	0.42
Significance (P value)		
Shading	0.698	0.244
Shoot hierarchy	-	<0.001
Shading x shoot hierarchy	-	0.912

Supplementary Table 1. Estimations of effective duration of grain filling (bilinear model parameter *c*) and rate of filling (bilinear model parameter *b*) (± 95% confidence interval) for grains at different positions on the ear; analysis of data by split-line regression for crops of spring barley either grown unshaded or shaded post-anthesis in 2011.

		Significance of fit		Estimated grain filling duration (days)		Estimated grain filling rate (mg/day)	
Grain Position	**Shading**	**R^2**	**P-value**	**Duration (±CI)**	**% change when shaded**	**Rate (±CI)**	**% change when shaded**
+1	Unshaded	0.95	<0.001	43.6 (± 2.32)	+5%	1.40 (± 0.135)	-25%
	Shaded	0.94	<0.001	45.9 (± 2.91)		1.05 (± 0.117)	
-1	Unshaded	0.96	<0.001	44.5 (± 2.28)	+4%	1.46 (± 0.135)	-23%
	Shaded	0.96	<0.001	46.3 (± 2.32)		1.13 (± 0.101)	
+6	Unshaded	0.95	<0.001	43.6 (± 2.32)	+2%	1.33 (± 0.127)	-26%
	Shaded	0.94	<0.001	44.6 (± 2.61)		0.99 (± 0.107)	
-6	Unshaded	0.95	<0.001	45.9 (± 2.51)	0%	1.39 (± 0.135)	-24%
	Shaded	0.95	<0.001	46.0 (± 2.67)		1.05 (± 0.109)	
+9	Unshaded	0.95	<0.001	43.1 (± 2.34)	+2%	1.24 (± 0.121)	-26%
	Shaded	0.94	<0.001	43.9 (± 2.51)		0.92 (± 0.095)	
-9	Unshaded	0.89	<0.001	43.7 (± 3.56)	0%	1.07 (± 0.158)	-25%
	Shaded	0.82	<0.001	43.7 (± 4.71)		0.80 (± 0.156)	
+12	Unshaded	0.92	<0.001	42.8 (± 2.77)	1%	1.06 (± 0.123)	-27%
	Shaded	0.84	<0.001	43.3 (± 4.35)		0.77 (± 0.137)	
-11	Unshaded	0.49	<0.001	42.0 (± 0.73)	-17%	0.67 (± 0.214)	-10%
	Shaded	0.46	<0.001	34.7 (± 6.08)		0.60 (± 0.279)	

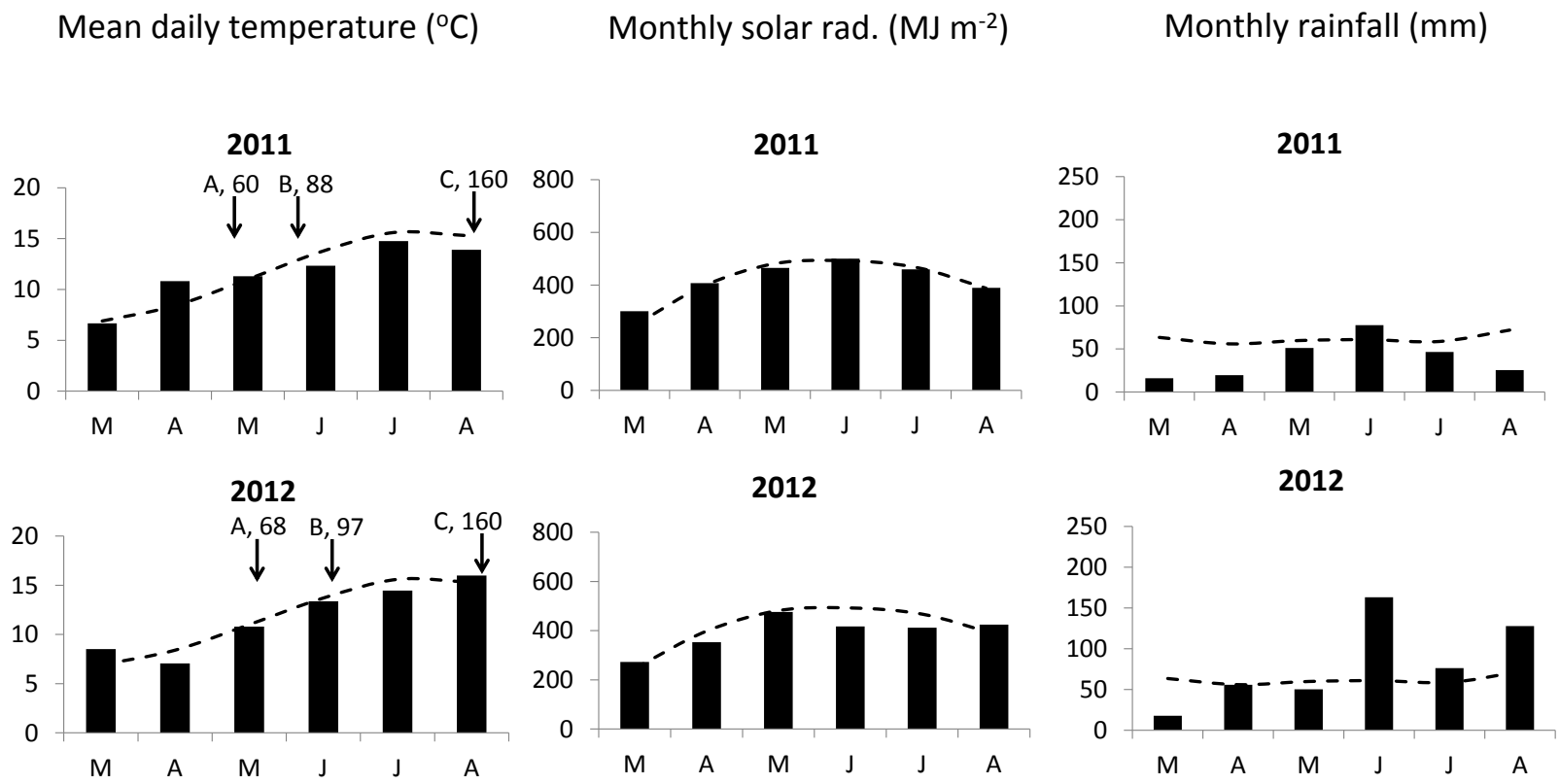

Figure 1. Mean monthly weather data for March to April at field sites in 2011 and 2012 (bars). Broken line gives the long-term average (2005-2012) for the site. In the mean daily temperature panels letters A-C refer to Zadoks growth stages 31, 55 and 92 respectively; numbers following the letter are the number of days after sowing the growth stage was reached.

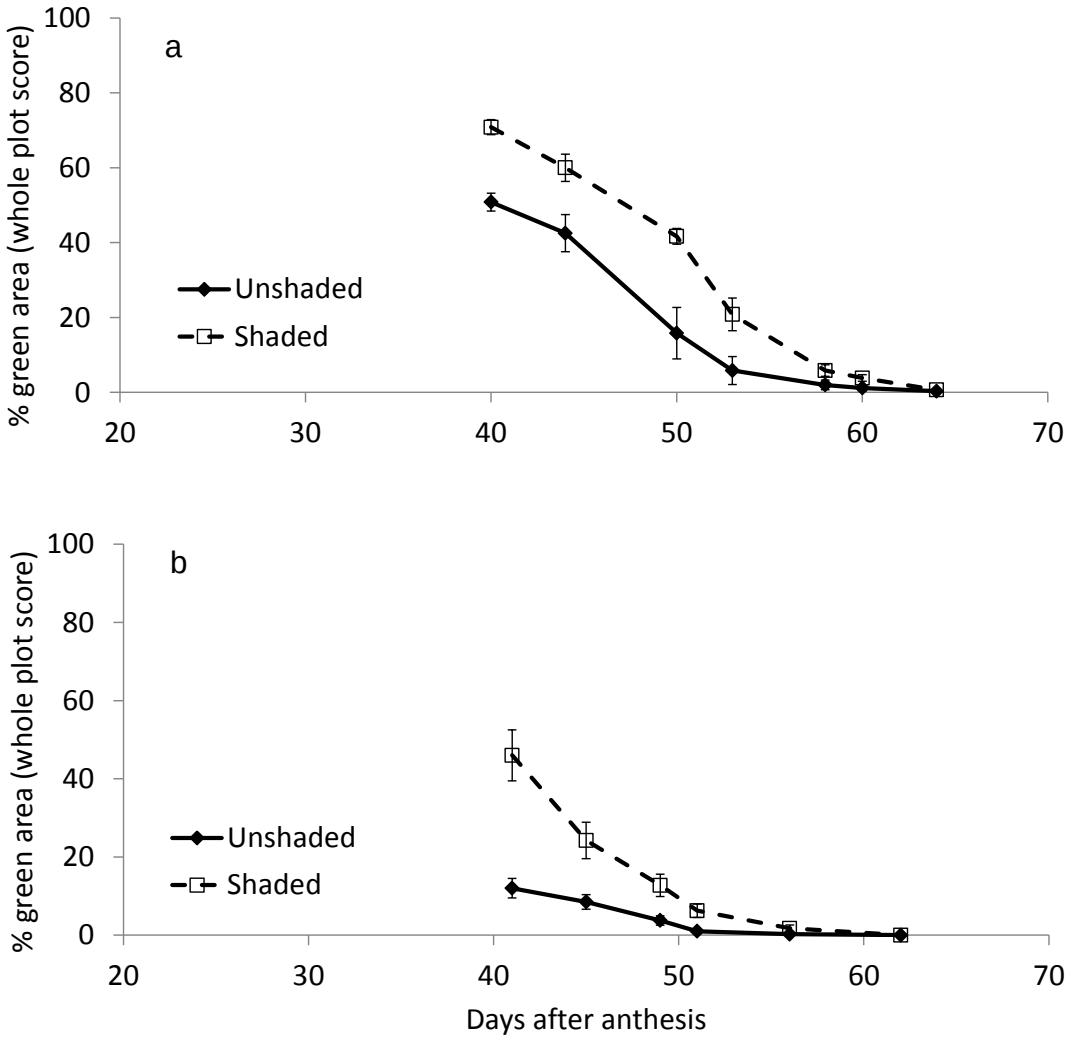

Figure 2. Canopy senescence for spring barley grown unshaded or shaded from GS 55/61 + 14 days until crop maturity in 2011 (a) and 2012 (b). Values are means ± SEM of % green area scored on a whole plot basis over the latter stages of senescence.

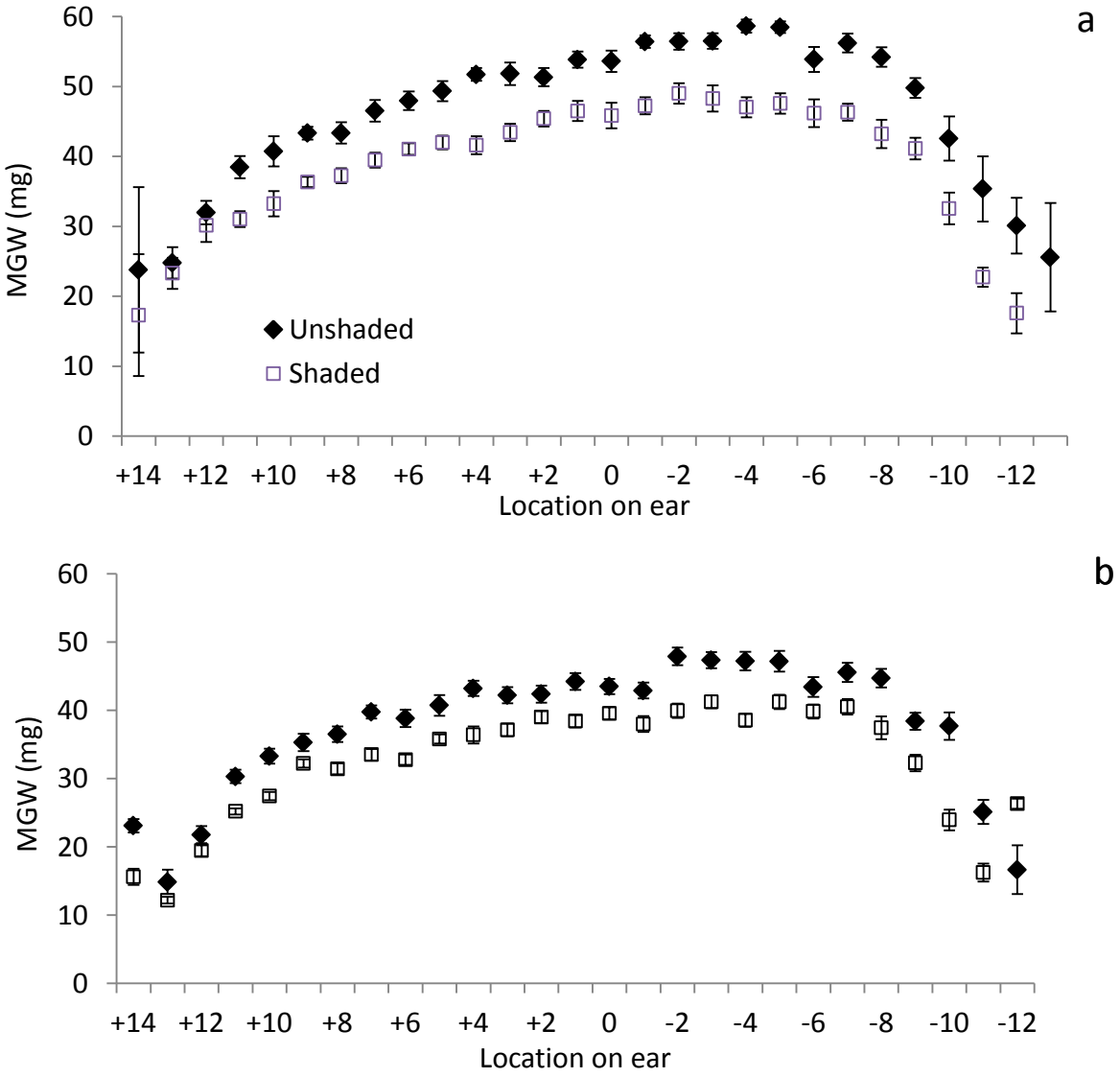

Figure 3. Plots of grain weight at individual spikelet locations on main stem ears at harvest for spring barley grown unshaded or shaded from GS 55/61 + 14 days until crop maturity in (a) 2011 and (b) 2012. Values are means ± SEM of ten sampled ears from each of 4-6 replicate plots, with locations referring to grains above (+) or below (-) the central spikelet on each ear. MGW includes the weight of the lemma and palea.

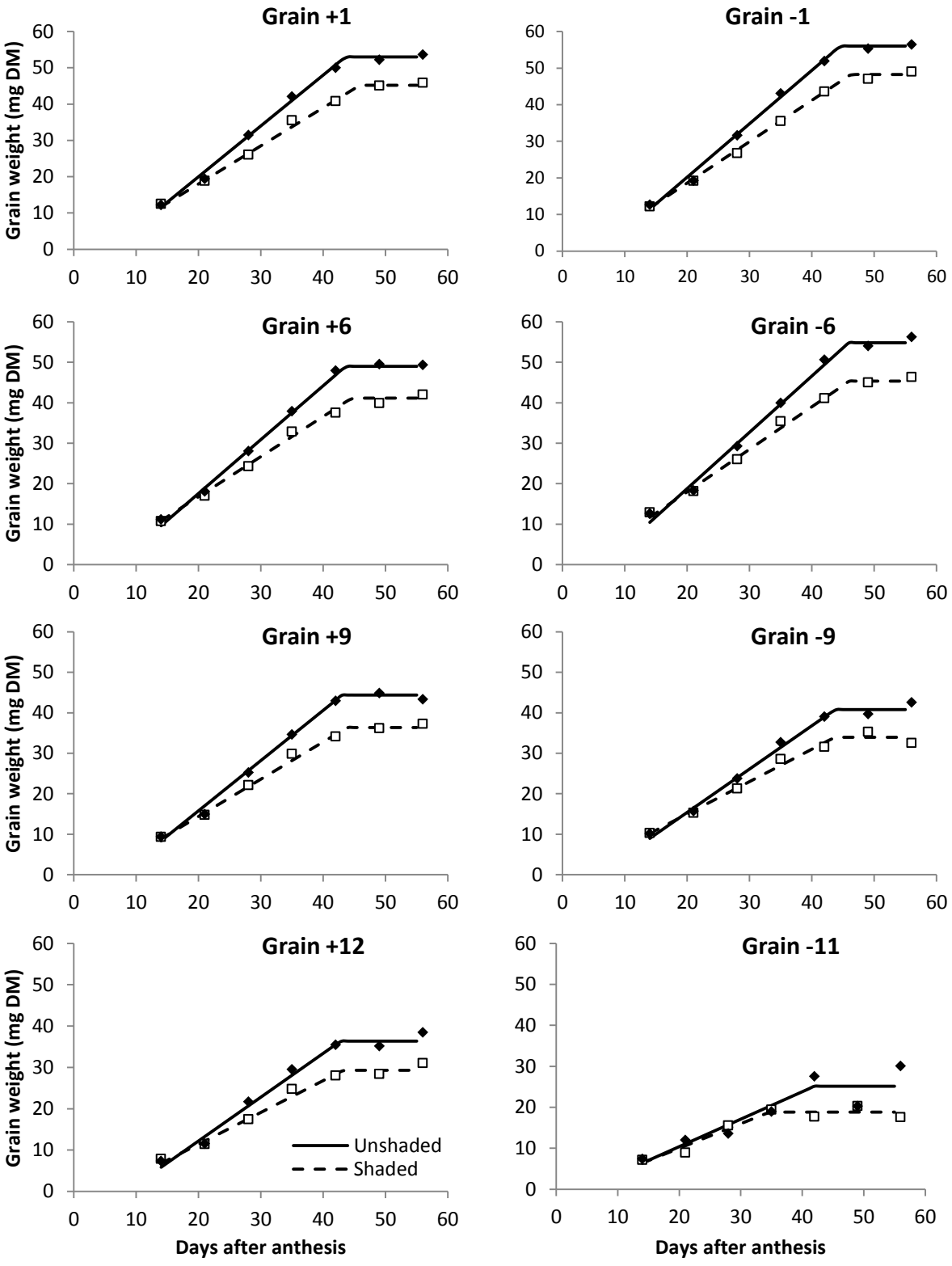

Figure 5. Split-line regression plots of the relationship between time after anthesis and the individual grain weight at specific spikelet positions on the main stem ear for spring barley crops grown in 2011 either unshaded or shaded from GS 55/61 + 14 days until crop maturity. At all time points grain weight includes the weight of the lemma and palea. Values are means of 10 ears from each of 6 replicate plots. Parameter values of the bilinear models are given in Table S1.

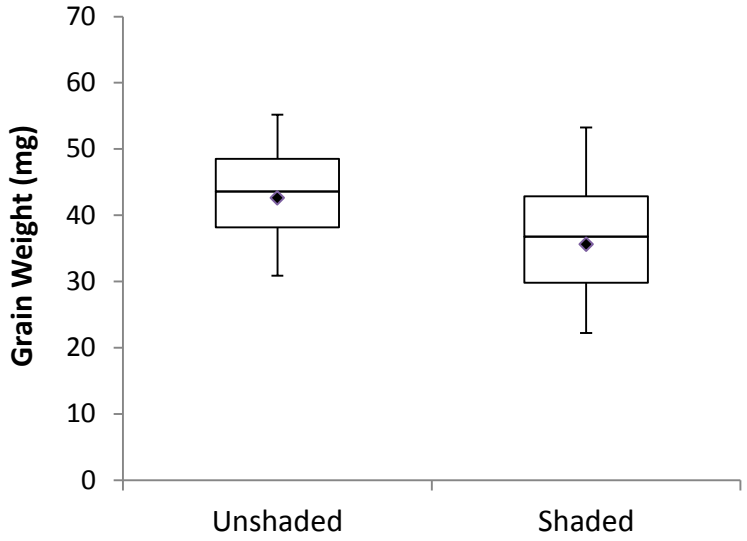

Figure 7. Box plot of individual grain weight in samples of 600 grains from spring barley crops harvested in 2011 grown either unshaded or shaded from GS55/61 + 14 days until crop maturity. Grains <5.0 mg were excluded from the analysis as non-viable. Boxes represent the central quartiles of data distribution whilst the ends of the whisker bars reflect the 90^{th} (upper) and 10^{th} (lower) percentiles respectively; horizontal line is the median value and the solid symbol the mean.

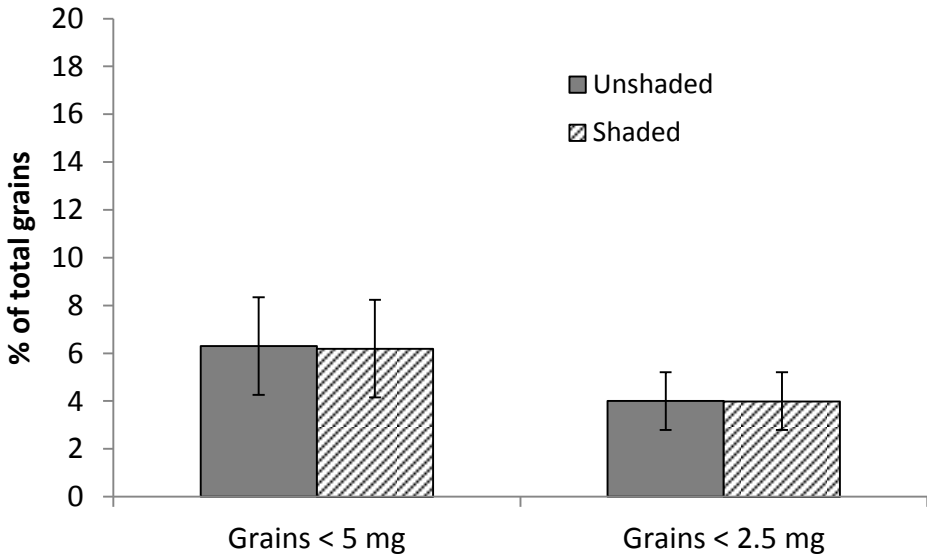

Figure 6. Proportion of grains weighing below 5 and 2.5 mg for spring barley crops harvested in 2011 either shaded from GS55/61 + 14 days until crop maturity, or grown unshaded. Error bars represent the standard error of the mean of 100-grain samples from six replicate plots.

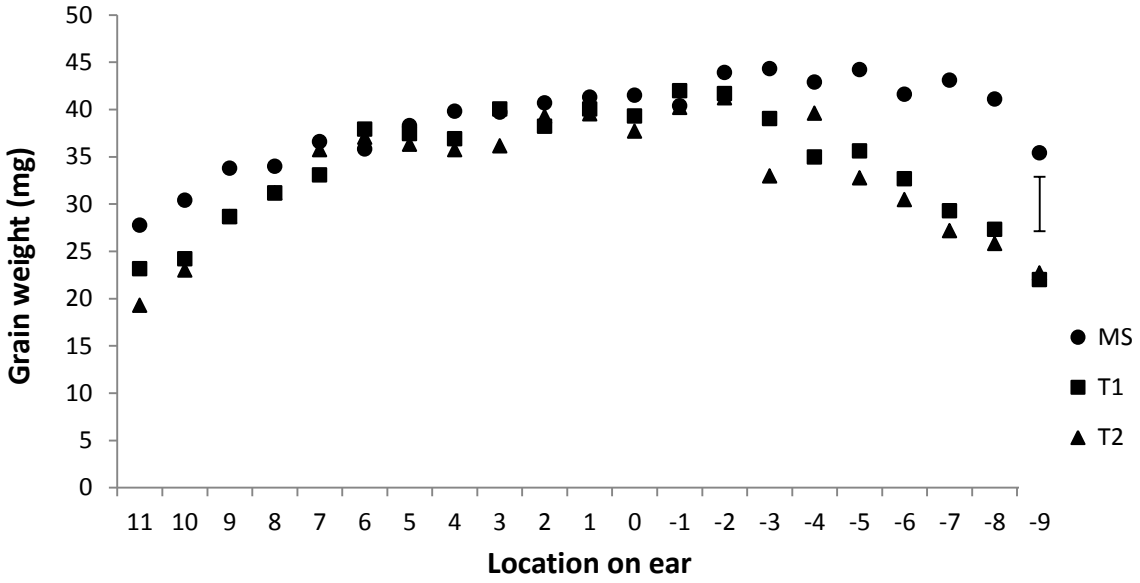

Figure 4. Grain weight on main shoot (MS), tiller 1 (T1) and tiller 2 (T2) ears at individual spikelet locations relative to the central spikelet (location 0). Data are means across the two shading treatments (unshaded, shaded) at harvest in 2012. Error bar is LSD 5% for the grain zone x tiller interaction effect following repeated measures ANOVA.

1

2

3